PATENT
PD1294

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re Application of
Grotendorst, et al.

Serial No.: 08/167,628
Filed : 12/14/93

FOR: CONNECTIVE TISSUE GROWTH FACTOR

Art Unit 1812

Examiner: L. Spector

FAX COPY
RECEIVED
JUL 5 1994
PTO MALL 1

DECLARATION UNDER 37 CFR §1.132

Commissioner of Patents
and Trademarks
Washington, D.C. 20231

Dear Sir:

We, Gary R. Grotendorst, Ph.D., and Douglass M. Bradham, Jr., Ph.D., declare and state that:

1. We are co-inventors of the subject matter described and claimed in the United States Patent Application Serial No. 08/167,628, filed on December 14, 1993, entitled, "Connective Tissue Growth Factor".

2. We are familiar with the prosecution history of Patent Application Serial No. 08/167,628 (FWC of 07/752,427, filed on August 30, 1991).

3. We understand that the Examiner has rejected claims 1 and 4 under 35 U.S.C. §102(b) as anticipated by or, in the alternative, under 35 U.S.C. §103 as obvious over Matsuoka, et al., or alternatively Campochiaro, et al., or alternatively Shinokado, et al.

4. As co-author on the Matsuoka, et al. reference (*Proc. Natl. Acad. Sci. USA*, 86:4416, 1989), Dr. Grotendorst has recently revieved the reference and it has become evident that there is a printer's error in the legend to FIGURE 4B. The error indicates that the mitogenic activity of the anti-PDGF immunoabsorbed fraction is indicated by the solid circles and the total wound fluid activity by the open circles. The opposite is correct. The legend for FIGURE 4C correctly states that the total chemotactic activity is indicated by the solid circles and the anti-PDGF immunopurified samples are the open circles. This makes the peak of anti-PDGF immunopurified mitogenic and chemotactic activities day 1, not day 4 or 5.

5. The mitogenic and chemotactic activities in Matsuoka correlated only with the 16-17 kD peptide(s), as shown in Figure 4, page 4419. The 34-36 kD fraction possessed no biological activity and was only present in trace amounts at the time when PDGF-like bioactivity was observed in wound fluid. Page 4418, column 2, lines 15-27, describes the correlation between the peptides described in the reference and biological activity. The kinetics of appearance and disappearance of the 16-17 kD and 34-36 kD product were independent of each other. According to the analysis of Figure 4B and 4C on page 4418, the Figure legend is mislabeled; the closed circles represent the total wound fluid and the open circles represent the immunopurified material. Importantly, as stated in the discussion on page 4418, column 2, lines 15-27, the level of the 16-17 kD peptide peaked on the first day after surgery and decreased exponentially to nearly undetectable levels by the seventh day. In contrast, the 34-36 kD product was initially present at low levels and then increased, reaching peak levels on the fifth and sixth days postsurgery. The chemotactic and mitogenic activity of the total wound fluid and immunoabsorbed

fraction show the highest activity on day 1 and decreased to undetectable levels by day 4, which correlates with the kinetics of appearance and disappearance of the 16-17 kD peptide.

6. Additional evidence that the 34-36 kDa fraction does not contain mitogenic activity is shown in the group of experiments shown in the accompanying EXHIBITS. The experiments shown were performed in the U.S., prior to the date of the Matsuoka reference (prior to June, 1989) by both of us (Dr. Grotendorst and Dr. Bradham) in our laboratory and under our supervision. The first experiment shows that the mitogenic activity present in the anti-PDGF IgG absorbed fraction after day 3 post surgery, at a time when the 34-36 kDa fraction is elevated, is the same as the negative control background indicating that there is no activity in the 34-36 kDa sample (SEE EXHIBIT A).

7. Briefly, anti-PDGF IgG purified wound fluid samples from days 1-5 post surgery were analyzed for mitogenic activity in a standard (^{3}H)-thymidine uptake assay for DNA synthesis (see Matsuoka, et al., for experimental methods). Mitogenic assays were performed using 48 well plates and NRK cells as target cells. The cells were plated in DMEM, 10% FCS and the 3T3 cells made quiescent by incubating for 2 days in serum-free DMEM containing ITS supplement (Collaborative Biomedical, Bedford, MA) before use. Varying amounts of the conditioned media containing the CTGF fractions and known amounts of recombinant PDGF standard were added to the wells and the plates incubated at 37°C in 10% CO_2, 90% air for 18 hours, after which ^{3}H-thymidine at a final concentration of 5 uC/ml was added and incubated for an additional 2 hours. The media was removed, the cells washed and DNA synthesis determined

from the ^{3}H-thymidine incorporation into trichloroacetic acid precipitable material by scintillation counting.

The cpm in the day 1 sample (see #30, 17374 cpm) is significantly above the background level of 2226 to 3886 cpm in samples #51-54. However, the samples from days 3-5 (#33-35), which is when the 34-36 kDa fraction peaks (See Figure 4 of Matsuoka), range from 1735 to 3671, which is essentially the same as background. Therefore, the fraction which correlates with the appearance of the 34-36 kDa peak in Matsuoka, et al., does not exhibit any mitogenic activity. (Samples 49-58 are standards containing different amounts of PDGF or, 49-50, FGF).

8. A second experiment examined chemotactic activity in the samples and indicated that none of the activity after day 3 could be neutralized with anti-PDGF IgG (SEE EXHIBIT B and Materials and Methods in Matsuoka, et al.). This indicates that the low level of activity is likely due to non-specific activation and that the 34-36 kDa protein fraction is not active as a chemoattractant.

9. Briefly, the ability of anti-PDGF IgG to neutralize the chemotactic activity present in the anti-PDGF IgG purified fraction (PDGF related) of human wound fluids collected on various days (D1=day 1, D2=Day 2, etc.) was examined. As seen in the column to the far right, only D1 shows significantly high activity which is able to be neutralized with anti-PDGF IgG. For example, sample 1 is Day 1 in the presence of non-immune IgG and activity is 157; sample 2 is the same material in the presence of anti-PDGF IgG had an activity of 28. Sample 19 is a 10 ng/ml PDGF standard in the presence of non-immune IgG and activity is 167; sample 20 is the same material in the presence of anti-PDGF IgG with an activity of 40.

Therefore, samples with activity below 28-40 have no PDGF-like activity. The 16-17 kDa fraction shown in Figure 4 of Matsuoka peaks on Day 1 post surgery, the time at which chemotactic activity is the highest (see D1). In contrast, at a time when the 34-36 kDa fraction peaks (day 4-5), there is no detectable chemotactic activity.

Therefore, the biological activity found in Matsuoka, et al., correlates with a 16-17 kD protein and not a 34-36 kD protein.

10. We understand that Claim 1 stands rejected under 35 U.S.C.-§102(a) as anticipated by or, in the alternative under 35 U.S.C.§103 as obvious over Ryseck, et al..

11. The clone and sequence of CTGF were obtained by both of us in our laboratory, in the United States, and submitted to GenBank on July 17, 1990, prior to the May 1991 publication date of Ryseck. The Office Action states that a comparison of the amino acid sequence of fisp-12 and CTGF reveals only 13 discrepancies in the region between 86 to 393. The Office Action states that there is greater divergence in the region preceeding residue 86. The Office Action states that Ryseck identifies this region as a signal sequence which would not affect protein activity.

12. We disagree with the conclusions stated in the Office Action. It is well known in the art that a typical signal sequence is about 15-25 amino acids in length. In fact, on page 227 of Ryseck, line 5, the authors state that the signal sequence of fisp-12 is only 21 amino acids (also see FIGURE 3). The cleavage site for the signal sequence is between residues 25 and 26 (page 226, column 2, second paragraph). Therefore, the sequence divergence found in amino acids 26-86 is significant

and therefore the fisp-12 protein described by Ryseck is distinguishable from CTGF of the present invention.

13. Further, prior to the May, 1991 date of the Ryseck reference, we had immunoaffinity purified CTGF and shown that it had mitogenic activity in a DNA synthesis assay using NRK fibroblasts. EXHIBIT C shows our laboratory notebook pages for experiments which were performed prior to the date of the Ryseck reference shewing that immunoaffinity purified CTGF has mitogenic activity.

14. Briefly, serum free (S/F) media from cultured HUVE cells was affinity purified on a column of Affi-Gel-10 conjugated with anti-PDGF IgG by methods described in Matsuoka, et al. (cited in this office Action). Affinity purified material was analysed in a Western blot and in a mitogenic assay using NRK cells as described by Matsuoka, et al. The data shown in EXHIBIT C, page 2 (table of cpm/sample) indicate that the affinity purified material (see for example samples 7-13) had mitogenic activity comparable to purified PDGF (samples 14-17).

Western blot analysis of the affinity purified mitogenic fractions revealed a protein with mitogenic activity that migrated at about 36 kDa. This protein fraction was identified as CTGF.

15. The identification of PDGF-like activity in HUVE cell conditioned media prompted the cloning and the isolation of a full length CTGF clone from a HUVE cell library (see Examples of the present patent application). The clone, designated DB60, was isolated from a HUVE cell cDNA library in λgt11 screened with anti-PDGF antibody (EXHIBIT D). Anti-PDGF antibody binding to the fusion protein produced by the clone DB60 was

completely blocked by the affinity purified proteins. A Northern blot analysis using RNA from HUVE cells indicated that the clone hybridized with a mRNA of about 2.4 kb, which is a message of sufficient size to produce a protein in the 38 kD molecular weight range as seen on the immunoblots of the affinity purified proteins.

The clone encoding the entire open reading frame of the CTGF protein was isolated in the U.S. prior to the May, 1991 date of the Ryseck reference.

16. We declare that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under § 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date July 2, 1994 Gary J. Grotendorst, Ph.D.

Date _____ Douglass M. Bradham, Ph.D.